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Efficacy of Teicoplanin-Gentamicin Given Once a Day on the Basis of Pharmacokinetics in Humans for Treatment of Enterococcal Experimental Endocarditis

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With the aim of investigating home therapy for enterococcal endocarditis, we compared the efficacy of teicoplanin combined with gentamicin given once a day or in three daily doses (t.i.d.) with the standard treatment, ampicillin plus gentamicin administered t.i.d., for treating experimental enterococcal endocarditis. The antibiotics were administered by using "human-like pharmacokinetics" (H-L), i.e, pharmacokinetics like those in humans, that simulated the profiles of these drugs in human serum. Animals with catheter-induced endocarditis were infected intravenously with 10^8 CFU of *Enterococcus faecalis* EF91 (MICs and MBCs of ampicillin, gentamicin, and teicoplanin, 0.5 and 32, 16 and 32, and 0.5 and 1 μg/ml, respectively) and were treated for 3 days with ampicillin H-L at 2 g every 4 h plus gentamicin H-L at 1 mg/kg every 8 h, or teicoplanin H-L at 10 mg/kg every 24 h, alone or combined with gentamicin, administered at dose of H-L at 1 mg/kg every 8 h or H-L at 4.5 mg/kg every 24 h. The results of therapy for experimental endocarditis due to EF91 showed that teicoplanin alone was as effective as ampicillin alone in reducing the bacterial load ($P > 0.05$). The combination of ampicillin or teicoplanin with gentamicin was more effective than the administration of both drugs alone in reducing the $\log_{10}$CFU/gram of aortic vegetation ($P < 0.01$ and $P < 0.05$, respectively). Teicoplanin plus gentamicin H-L at 4.5 mg/kg, both administered every 24 h, showed an efficacy equal to the "gold standard," ampicillin plus gentamicin H-L at 1 mg/kg t.i.d. ($P > 0.05$). Increasing the interval of administration of gentamicin to a single daily dose combined with teicoplanin resulted in a reduction of bacteria in the vegetations equivalent to that achieved with the recommended regimen of ampicillin plus thrice-daily gentamicin in the treatment of experimental endocarditis due to *E. faecalis*. Teicoplanin plus gentamicin, both administered once a day, may be useful home therapy for selected cases of enterococcal endocarditis.

The combination of penicillin or ampicillin and an aminoglycoside during 4 to 6 weeks is the recommended therapy for enterococcal endocarditis (2). The daily dose of aminoglycoside has generally been administered to patients in three, or ocassionally, two divided doses. However, to avoid the potential toxic effects of prolonged aminoglycoside therapy, new treatment regimens, such as increasing the dosing interval of aminoglycoside administration, have been studied. Moreover, pharmacodynamic data obtained from the animal model (22, 23, 39) have shown that the aminoglycoside exhibit concentration-dependent killing, supporting the idea that once-a-day administration might be more efficacious. Along this line, the efficacy of gentamicin given once a day has been shown to be effective for the treatment of experimental endocarditis due to viridans streptococci (3, 16, 34) and due to *Staphylococcus aureus* (J. Gavaldà, M. Laguarda, J. A. Capdevila, L. Pou, E. Crespo, and A. Pahissa, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. B44, 1995). However, the efficacy of once-a-day gentamicin in the treatment of experimental endocarditis due to *Enterococcus faecalis* remains controversial (11, 17, 21, 26, 36).

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The possibility of home therapy for endocarditis due to viridans streptococci using a combination of ceftriaxone plus an aminoglycoside, both administered once a day, has been evaluated in animal models of endocarditis (3; C. W. Dorscher, B. M. Tallan, M. S. Rouse, J. M. Steckelberg, H. C. Chambers, and W. R. Wilson, 30th ICAAC, abstr. 700, 1990; H. F. Chambers, S. Kennedy, M. Fournier, M. Rouse, and W. Wilson, 30th ICAAC, abstr. 701, 1990) and in humans (14). This therapeutic approach was also demonstrated to be effective in experimental studies of endocarditis due to *Staphylococcus aureus* (Gavaldà et al., 35th ICAAC).

The glycopeptides vancomycin and teicoplanin may be useful alternatives to penicillin for treating enterococcal endocarditis in cases of resistance, allergy to penicillins, or for outside-hospital treatment. Teicoplanin has an antibacterial spectrum similar to that of vancomycin, and it has a longer elimination half-life, which allows once-a-day intravenous (i.v.) or intramuscular administration. These features offer the chance for home treatment in selected cases of infective endocarditis.

The aim of the present study was to evaluate the efficacy of teicoplanin combined with gentamicin, both given once a day, in the treatment of experimental endocarditis due to *E. faecalis*. The antibiotics were administered to the test animals by means of a pharmacokinetic model that mimicked the human serum profile of the drugs.

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MATERIALS AND METHODS

Strains, media, and antimicrobials. We studied seven *E. faecalis* strains susceptible to ampicillin, teicoplanin, and aminoglycosides, originally isolated from patients with endocarditis, and *E. faecalis* ATCC 29212 as reference test strain. All the strains were identified by the API 20 STREP system (bioMérieux, La Balme Les Grottes, France) and confirmed according to the criteria recommended by Facklam and Collins (10). The in vivo studies were performed with *E. faecalis* EF91. Working stock cultures were kept frozen at −70°C in double-strength skim milk (Difco Laboratories, Detroit, Mich.). Before each experiment, one aliquot was thawed and subcultured onto 5% sheep blood Columbia agar plates (bioMérieux).

Mueller-Hinton broth (MHB; Difco Laboratories), 5% sheep blood Mueller-Hinton agar plates, and 5% sheep blood Columbia agar plates (BCA; bioMérieux) were used. The antibiotics studied were ampicillin (Antibioticos S.A., Madrid, Spain), teicoplanin (Aventis S.A., Madrid, Spain), and gentamicin (Sigma Chemical Co., St. Louis, Mo). Ampicillin and teicoplanin solutions were prepared fresh on the day used. Stock solutions of gentamicin were prepared and stored at −70°C.

In vitro studies. MICs were determined on cation-adjusted MHB by the standard microdilution method following the guidelines of the NCCLS (30). The MBC was determined after 48 h of incubation of 0.025 ml from the control well, from the first well containing growth, and from all the wells without visible growth plated onto 5% BCA plates for colony count. The MBC was defined as the lowest concentration of antibiotic that killed 99.9% of the original inoculum (1).

To perform time-kill synergy studies, we followed the method described by Sahm and Torres (34). Prior to inoculation, each tube of fresh, prewarmed MHB was supplemented with gentamicin (final concentrations, 1, 3, and 10 μg/ml) either alone or in combination with teicoplanin (final concentrations, 0.5 and 10 μg/ml). A positive growth tube without antibiotics was used as a control. Test tubes were inoculated with 10^7 CFU of *E. faecalis*/ml in the logarithmic phase of growth, incubated at 37°C in room air, and the $\log_{10}$ of the number of CFU/milliliter was determined after 0, 4, and 24 h of incubation. Synergy was defined as a ≥ 2 $\log_{10}$ decrease in the CFU/milliliter between the combination and its most active agent alone after 24 h, and the number of surviving organisms in the presence of the combination had to be ≥ 2 $\log_{10}$ CFU/ml below the starting inoculum. The combination was considered to have bactericidal activity when a ≥ 3-$\log_{10}$ reduction in colony counts was reached.

Animals. New Zealand White rabbits (body weight, 2 kg) were obtained from BK Universal (Barcelona, Spain). The animals were housed in the animal facility of our hospital, which is equipped with automatic air exchange with a HEPA filter and a circadian light cycle. The animals were nourished ad libitum with sterile water and feed.

Pharmacokinetics. Ampicillin, teicoplanin, and gentamicin were administered using a system to reproduce the human serum pharmacokinetics in rabbits and mimic the human serum profile after an i.v. infusion of ampicillin (2 g/4 h), teicoplanin (10 mg/kg/24 h), gentamicin (1 mg/kg/8 h), or gentamicin (4.5 mg/kg/24 h). We employed a computer-controlled infusion pump system that delivered decreasing quantities of drug (Alice King). The computer software was written by our group. With this program the flow rate of the pump can be changed automatically, and flow rate sequences can be repeated as often as required. This approach involved three steps: (i) estimation of ampicillin, teicoplanin, and gentamicin pharmacokinetic parameters in the rabbit; (ii) application of a mathematical model to obtain the required infusion doses to simulate human kinetics in the animals; and (iii) in vivo experimental pharmacokinetic studies. These studies were done to simulate in rabbits the pharmacokinetic profile of ampicillin, teicoplanin, and gentamicin in humans.

The pharmacokinetic studies which led to the human-like pharmacokinetics of ampicillin in rabbits, including the explanation of the mathematical model to use on the basis of an open one-compartment model, have been described previously (17). The pharmacokinetic data of the human-adapted model of 2 g of ampicillin given i.v. in rabbits are shown in Table 1 and Fig. 1A. The serum profile and the pharmacokinetic parameters of ampicillin in rabbits administered antibiotics with this model were similar to those of 2 g of i.v. ampicillin in humans (17).

(i) Estimation of teicoplanin and gentamicin pharmacokinetic parameters in rabbits. To determine the concentrations of teicoplanin in serum, blood was drawn from a carotid catheter at 4, 8, 10, 15, 20, 30, and 60 min and at 2, 4, and 6 h after a single i.v. injection of a 20-mg/kg dose of teicoplanin. This study was done in four healthy rabbits. Teicoplanin concentrations were determined by an immunoassay method (TDx; Abbott Diagnostics, Irving, Tex.). The sensitivity of the assay was 1.8 μg/ml of sample, and the between-and within-day coefficients of variation for replicates ($n = 7$) at 4 and 80 μg/ml were <5%.

To determine concentrations of gentamicin in serum, blood was drawn from a carotid catheter at 4, 10, 15, 20, 25, 30, 45, 60, and 90 min and at 2, 2.5, 3, 3.5, and 4 h after a single i.v. injection of a 6-mg/kg dose of gentamicin. This study was done in seven healthy rabbits. Gentamicin concentrations were determined by an immunoassay method (Abbott). The sensitivity of the assay was 0.25 μg/ml of sample, and the between-and within-day coefficients of variation for replicates ($n = 7$) were <4.6% and 2.5%, respectively.

The serum disposition constants (ær, βr), the zero-time intercept for the æ phase (*A*r) and the zero-time intercept for the β phase (*B*r) of teicoplanin and gentamicin in the rabbit were determined by using a nonlinear least-squares regression analysis of the concentration-in-serum-versus-time curve on the basis of an open two-compartment model.

(ii) Application of a mathematical model to obtain the required infusion doses to simulate the human kinetics of teicoplanin and gentamicin in both dosages as drugs with an open two-compartment model. The mathematical model dealing with the administration of drugs with human-like pharmacokinetics on the basis of an open two-compartment model has been previously published (18).

(iii) In vivo experimental pharmacokinetic studies. In vivo pharmacokinetic studies were done to simulate in rabbits the pharmacokinetic profile of a 10-mg/kg dose of teicoplanin or gentamicin given at 1 mg/kg/8 h and 4.5 mg/kg/24 h in humans. The doses of teicoplanin and gentamicin administered in three daily doses (t.i.d.) are those recommended for the treatment of endocarditis. Briefly, two polyethylene catheters (inner diameter, 0.81 mm; outer diameter, 1.27 mm; Portex SA; Hythe, Kent, England) were inserted, one through the carotid artery (sampling) and the other into the cava vein through the jugular vein (infusion), as previously described (17, 18). The pump system was set up to deliver previously calculated flow rates of i.v. infusion to simulate the human kinetics of teicoplanin or gentamicin administered t.i.d. or once daily. This study was done in four healthy rabbits for teicoplanin and in nine healthy rabbits each for gentamicin at doses of 1 mg/kg/8 h and 4.5 mg/kg/24 h. To determine serum teicoplanin levels, 2 ml of blood was sampled through the carotid catheter at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after the start of infusion. Teicoplanin

TABLE 1. Comparison of the pharmacokinetic parameters of the antimicrobials used in the in vivo studies[a]

Antimicrobial (dose) and subject	k_{el} (h^{-1})	$t_{1/2}$ (h)	$AUC_{0\rightarrow\infty}$ (μg · h/ml)
Teicoplanin (10 mg/kg)			
Rabbit[b]	0.7 ± 0.02	4.12 ± 0.6	296.3
Human[c]	0.068	10.1	731.7
Rabbit "human-like"[d]	0.061 ± 0.002	11.3 ± 0.4	765.3 ± 35.1
Gentamicin (1 mg/kg)			
Rabbit[b]	0.64 ± 0.22	1.09 ± 0.3	9.6
Human[c]	0.27	2.54	18
Rabbit "human-like"[d]	0.27 ± 0.034	2.5 ± 0.37	15.80 ± 1.24
Gentamicin (4.5 mg/kg)			
Rabbit[b]	0.64 ± 0.22	1.09 ± 0.3	24.05
Human[c]	0.25	2.8	66.2
Rabbit "human-like"[d]	0.24 ± 0.014	2.9 ± 0.18	76.84 ± 17.7
Ampicillin (2 g)			
Rabbit[b]	2.4 ± 0.29	0.3 ± 0.03	38.19
Human[c]	0.63	1.1	127.8
Rabbit "human-like"[d]	0.71 ± 0.05	0.99 ± 0.08	116.7 ± 31.83

[a] The pharmacokinetic parameters were estimated on the basis of an open one-compartment model.

[b] $t_{1/2}$ and k_{el} data are for healthy rabbits treated i.v. with a 20-mg/kg dose of teicoplanin, a 6-mg/kg dose of gentamicin, and a 100-mg/kg dose of ampicillin given i.v. The $AUC_{0\rightarrow\infty}$ was calculated from the data of an ideal profile obtained with a C_0 of 150 μg/ml for teicoplanin and 80 μg/ml for ampicillin, a *C* at 0.5 h of 5 μg/ml for gentamicin at 1 mg/kg, a *C* at 0.5 h of 18 μg/ml for gentamicin at 4.5 mg/kg, and the k_{el} of teicoplanin, ampicillin, and gentamicin in the rabbit.

[c] k_{el}, $t_{1/2}$, and $AUC_{0\rightarrow\infty}$ values were obtained from an ideal human serum profile of 10 mg of teicoplanin per kg, 2 g of ampicillin, and 1 or 4.5 mg of gentamicin per kg.

[d] Data are for H-L of 10 mg of teicoplanin per kg, 2 g of ampicillin, and 1 or 4.5 mg of of gentamicin per kg.

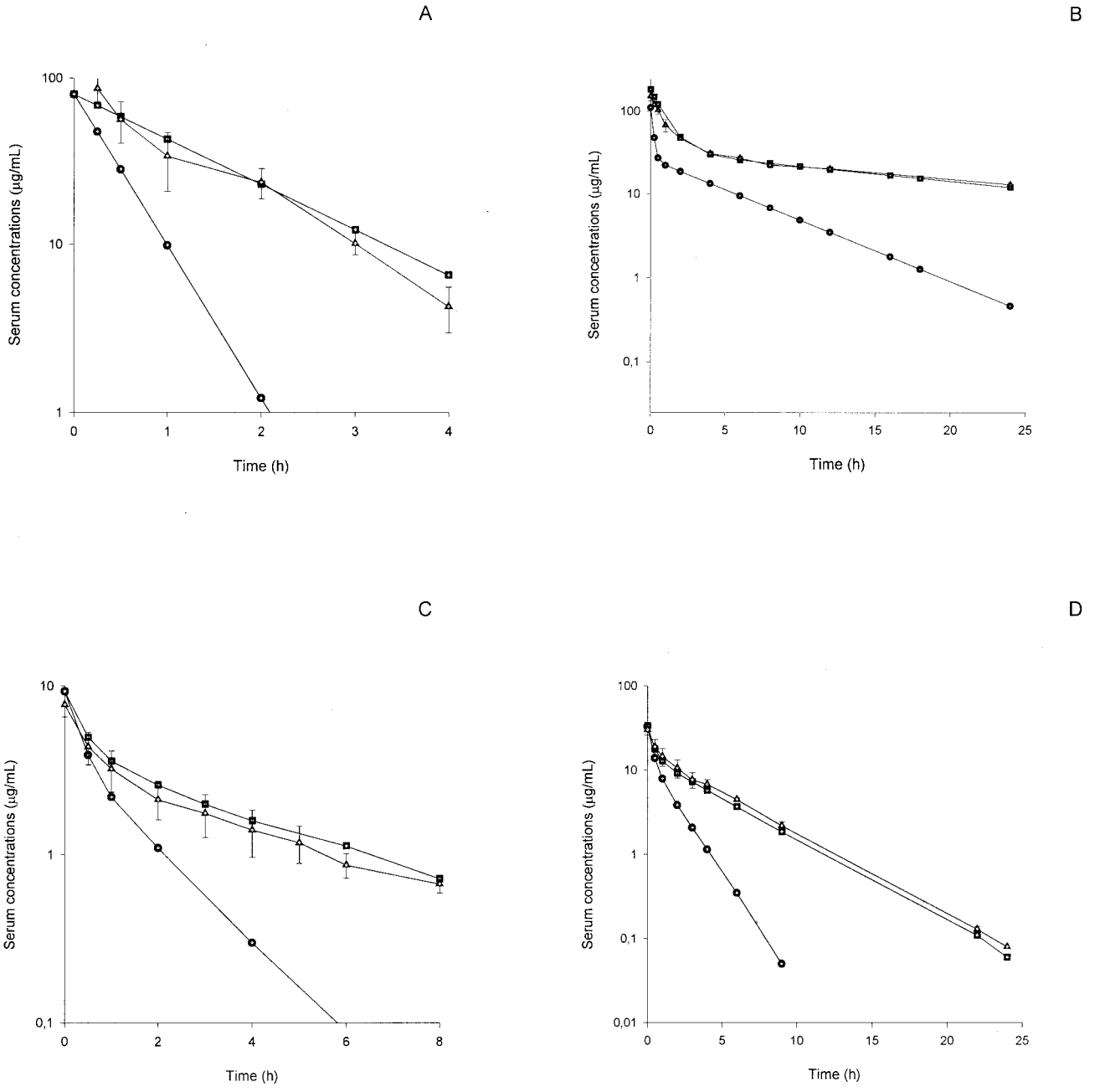

FIG. 1. Results of the pharmacokinetic studies in rabbits using H-L of 2 g of ampicillin (A), teicoplanin at 10 mg/kg (B), gentamicin at 1 mg/kg (C), and gentamicin at 4.5 mg/kg (D). Symbols: ○, rabbit ideal; □, human; △, rabbit "human-like."

concentrations in serum were assayed using the immunoassay described above. To estimate gentamicin human-like pharmacokinetics, 2 ml of blood was sampled at 0.1, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after the start of infusion in the rabbits mimicking the human profile of 1 mg/kg/8 h and at 0, 0.1, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 22, and 24 h in the animals mimicking the human profile of 4.5 mg/kg/24 h. Concentrations in serum were assayed as mentioned above. Different pharmacokinetic parameters were estimated on the basis of an open one-compartment model to compare the pharmacokinetics of teicoplanin and gentamicin in rabbits, in the human-adapted model, and in humans. The half-lives at the β phase of teicoplanin and gentamicin in the rabbits with human-like pharmacokinetics ($t_{1/2}$) were calculated as the ln $2/K_{el}$, where k_{el} is the elimination rate constant. The elimination rate constant was determined as the slope obtained from a linear regression analysis of the terminal phase of the plasma-concentration-versus-time curve on the basis of an open one-compartment model. Thereafter, the $AUC_{0\rightarrow\infty}$ was calculated as C_0/k_{el}, the pharmacokinetic parameters of teicoplanin in humans and rabbits were calculated as described above with a C_0 of 150 μg/ml; the pharmacokinetic parameters of gentamicin administered at dose of 1 mg/kg/8 h in humans and rabbits were calculated as described above with a $C_{0.5}$ of 5 μg/ml, and the pharmacokinetc parameters of gentamicin administered at dose of 4.5 mg/kg/24 h were calculated as described above with a $C_{0.5}$ of 18 μg/ml.

Establishing endocarditis and installing the infusion pump system. Experimental aortic valve infective endocarditis was induced in New Zealand White rabbits (weight, ca. 2 to 2.1 kg) by the method of Garrison and Freedman (15), modified as described by Durack et al. and Perlman and Freedman (6–9, 31, 32). The induction of non-bacterial thrombotic endocarditis was done as previously described (16, 17). Briefly, a polyethylene catheter was inserted through the right carotid artery into the left ventricular cavity and was left in place throughout the experiment. The same day, one or two catheters (inner diameter, 0.81 mm; outer diameter, 1.27 mm; Portex SA), depending on the treatment group, were placed

into the inferior cava vein through the jugular vein by the same technique described previously (18) to administer the ampicillin, teicoplanin, and gentamicin treatment. The infusion pump system was set up to deliver 2 ml of 0.9% saline per h to keep the catheter open until the beginning of antimicrobial administration. At 24 h after placement of the intracardiac catheter, different groups of animals were inoculated via the jugular catheter with 1 ml of saline containing 10^8 CFU of the *E. faecalis* EF91 strain in a stationary phase of growth. A 1-ml amount of blood was obtained 48 h after infection and just before initiation of antimicrobial therapy in order to confirm the presence of endocarditis. The blood speciment was mixed with 20 ml of molten Trypticase soy agar. Plates were incubated for 48 h at 35°C in room air, and the presence of enterococci was interpreted as indicative of infective endocarditis.

Treatment groups and estimation of therapeutic efficacy. Antimicrobial therapy was initiated 48 h after infection and was continued for 3 days. The infected rabbits were randomized into the following treatment groups: control without treatment; ampicillin "human-like pharmacokinetics" (H-L) at 2 g/4h i.v.; ampicillin H-L at 2 g/4 h i.v. plus gentamicin H-L at 1 mg/kg/8 h i.v.; teicoplanin H-L at 10 mg/kg/24 h i.v.; teicoplanin H-L at 10 mg/kg/24 h i.v. plus gentamicin H-L at 1 mg/kg/8 h i.v.; and teicoplanin H-L at 10 mg/kg/24 h i.v. plus gentamicin H-L at 4.5 mg/kg/24 h i.v.

After 3 days of treatment, the surviving animals were sacrificed 6 h after ending the infusion of antibiotics with a lethal i.v. injection of sodium pentothal. The chest was opened, the heart was excised and opened, and the aortic valve vegetations were removed aseptically. Only those animals with proper placement of the catheter, macroscopic evidence of vegetations at the time of sacrifice, and enterococci on cultures of blood obtained before the start of antimicrobial therapy were included in the study. The animals included in the control group were sacrificed 48 h after the induction of infection. The vegetations were rinsed with saline, weighed, and homogenized in 2 ml of Trypticase soy broth (Difco Laboratories) in a tissue homogenizer (Stomacher 80). Homogenates were quantitatively cultured onto 5% BCA plates. The plates were incubated for 48 h at 37°C in room air. In order to surmount the carryover effect, the vegetations of the animals treated with ampicillin or teicoplanin combined with gentamicin were washed by centrifugation of the homogenates at $2.500 \times g$ for 10 min. The supernatant was removed, and the bacterial pellet was resuspended in drug-free medium. We used two washings to ensure extensive drug removal. The homogenates of the vegetations from animals treated with ampicillin or teicoplanin alone were not washed because we needed a 10^{-3} dilution in order to obtain a culture that could be counted. Results were expressed as the $\log_{10}$ CFU of *E. faecalis* EF91 per gram of vegetation. Bacterial densities in valvular vegetations, calculated to be between 0 and 2 log CFU/g, were reported as $\log_{10}$ 2 CFU/g rather than 0 because of potential errors associated with the low weight of the valvular tissue.

Analysis of results. Results were expressed as the mean and the 95% confidence interval of the mean of the $\log_{10}$ CFU of *E. faecalis* per gram of vegetation. Differences in $\log_{10}$ CFU of enterococci per gram of vegetation were compared using one-way analysis of variance. When the F value was significant, each treatment group was compared with the control group and with each of the other treatment groups using Scheffe's test. P values of ≤ 0.05 were considered significant.

RESULTS

In vitro studies. The MIC and MBC of ampicillin, gentamicin, and teicoplanin for strain EF91 were 0.5 and 32, 16 and 32, and 0.5 and 1 μg/ml, respectively. The ranges of MICs and MBCs for ampicillin, gentamicin, and teicoplanin of the other strains were 0.5 to 1 and 8 to 32, 8 to 16 and 16 to 64, and 0.25 to 0.5 and 1 to 4 μg/ml, respectively. All strains were susceptible to ampicillin and teicoplanin, and no strains showed high-level resistance to gentamicin.

The results of the time-kill studies are shown in Table 2. Teicoplanin or gentamicin alone, at the concentrations tested, were only bacteriostatic (the viable counts of the culture did not decrease ≥ 3 $\log_{10}$ after 24 h of incubation). In contrast, the combinations of teicoplanin at 10 μg/ml and gentamicin at 1, 3, or 10 μg/ml showed a bactericidal effect. In four of seven strains the combination of teicoplanin at 0.5 μg/ml plus gentamicin lost this effect. After 24 h of incubation, synergy was present between teicoplanin plus gentamicin in all seven strains of *E. faecalis*. Figure 2 shows the time-kill curve of the strain used in the in vivo studies. The results of the combinations of teicoplanin at 10 μg/ml and gentamicin at 1, 3, or 10 μg/ml did not differ substantially from those shown in Fig. 2 for the combinations of telcoplanin at 0.5 μg/ml plus gentamicin at the different concentrations.

TABLE 2. Results of time-kill experiments at 24 h with seven *E. faecalis* strains

Antimicrobial agent(s)[b] (dose [μg/ml])	*E. faecalis* count (change in $\log_{10}$ CFU/ml)[a]						
	EF6	EF37	EF42	29212	EF105	EF69	EF91
Growth control	+1.97	+2.14	+2.26	+2.26	+1.72	+2.49	+1.91
T (0.5)	−0.47	+1.37	−1.32	−0.19	−0.3	+2.33	+0.67
T (10)	−1.06	+0.5	−2.1	+0.14	−0.2	−0.19	−0.71
G (1)	+1.68	+2.02	+2	+1.79	+1.77	+1.6	+1.64
G (3)	+1.39	+1.8	+1.88	+0.41	+1.04	+2.09	+1.45
G (10)	+0.18	+0.1	+0.1	−1.25	+0.18	−1.43	+0.78
T (0.5) + G (1)	−3.05	−3.1	−3.2	−2.9	−1.75	+2.2	−4.19
T (0.5) + G (3)	−3.14	−3.3	−3.3	−3.01	−2.93	+2.0	−4.82
T (0.5) + G (10)	−3.19	−4.1	−3.89	−3.55	−3.22	−0.84	−4.82
T (10) + G (1)	−3.2	−4.2	−4.15	−3.23	−3	−4.27	−3.87
T (10) + G (3)	−3.35	−4.98	−4.61	−4.22	−3.08	−4.38	−4.52
T (10) + G (10)	−3.68	−5.37	−5.21	−5.25	−3.06	−4.7	−4.82

[a] Results are given as the change of $\log_{10}$ CFU/ml with respect to the initial inoculum.
[b] T, teicoplanin; G, gentamicin.

Pharmacokinetic studies. The rabbit pharmacokinetic data of teicoplanin, determined in four healthy rabbits that had received one i.v. bolus dose of 20 mg/kg were as follows (mean ± standard deviation): ær, 7.85 ± 3.57 h^{-1}; βr, 0.1712 ± 0.02 h^{-1}; k_{21}r, 1.093 ± 0.52 h^{-1}; k_{13}r, 1.35 ± 0.54 h^{-1}; and Vr, 0.33 ± 0.14 liters/kg. The concentration profile in human serum produced by a 10-mg/kg i.v. injection of teicoplanin was successfully simulated in rabbits using the controlled-infusion pump system (Fig. 1B). The pharmacokinetic parameters obtained from the human-adapted model were similar to those of the 10-mg/kg i.v. dose teicoplanin in humans (Table 1).

The pharmacokinetic data of the 6-mg/kg dose of gentamicin given i.v. in nine healthy rabbits were as follows: ær, 2.96 ± 0.86 h^{-1}; βr, 0.6 ± 0.13 h^{-1}; k_{21}r, 1.49 ± 0.23 h^{-1}; k_{13}r, 1.14 ± 0.12 h^{-1}; and Vr, 0.2 ± 0.05 liters/kg. The results of the human-adapted model in rabbits, reproducing the pharmacokinetic parameters of the i.v. administration of 1- and 4.5-mg/kg doses of gentamicin in humans, are shown in Table 1 and Fig. 1C and the i.v. D.

Treatment of established endocarditis. Results of therapy for experimental endocarditis caused by *E. faecalis* EF91 are shown in Table 3. After 3 days of treatment, the bacterial counts were reduced in the vegetations of the treated animals compared to the control group with all the drug regimens ($P < 0.001$). Comparisons between the treated groups revealed that teicoplanin alone was as effective as ampicillin alone ($P > 0.05$). The combinations of gentamicin plus ampicillin or teicoplanin were significantly more effective than ampicillin alone in reducing the number of bacteria in the vegetations ($P < 0.01$). Teicoplanin plus gentamicin given once or daily t.i.d. were more efficacious than teicoplanin given alone ($P < 0.01$).

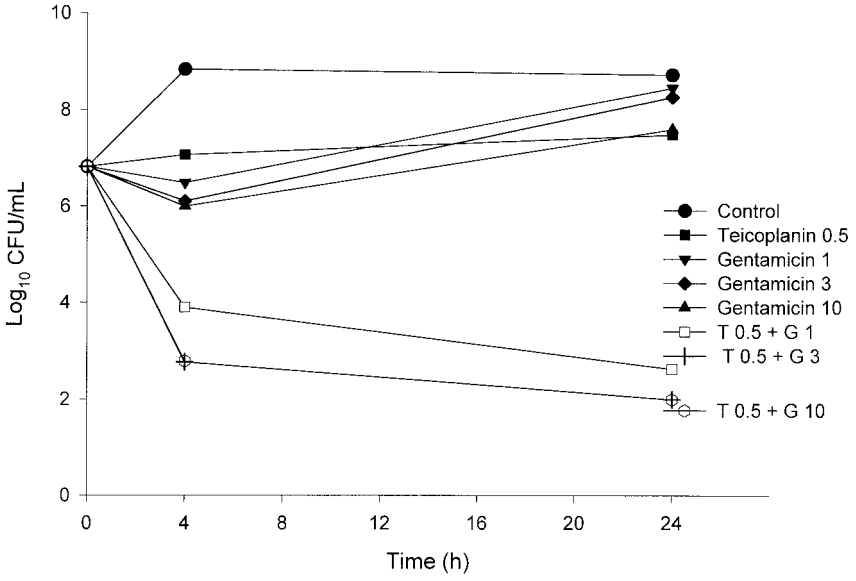

FIG. 2. Time-kill curve of teicoplanin (T) at 0.5 μg/ml and of gentamicin (G) at 1, 3, and 10 μg/ml for *E. faecalis* EF91.

Teicoplanin plus gentamicin H-L at 4.5 mg/kg, both administered once a day, was effective and displayed an activity similar to the gold standard, ampicillin plus gentamicin H-L at 1 mg/kg t.i.d. ($P > 0.05$).

DISCUSSION

The purpose of this study was to evaluate the efficacy of teicoplanin plus gentamicin, both given once a day and administered as H-L, for the treatment of experimental enterococcal endocarditis. Our results show that a single daily dose of gentamicin combined with teicoplanin was as effective as the recommended treatment for *E. faecalis* endocarditis in humans, which consists of ampicillin plus gentamicin at a dose of 1 mg/kg every 8 h.

Munckhof et al. (29) conducted a meta-analysis that evaluated all the randomized clinical studies dealing with once-a-day administration of aminoglycosides. They found that aminoglycosides can be given once daily, rather than in divided doses, for treating human infections other than endocarditis without loss of efficacy or increased toxicity, offering greater simplicity and potentially improved cost-effectiveness.

The advantage of once-daily administration of aminoglycosides for the treatment of endocarditis is presently under discussion (3, 5, 11, 16, 17, 35; Dorscher et al., 30th ICAAC). The combinations of aminoglycosides administered once-a-day with penicillin or ceftriaxone have been found to be effective in studies using pencillin-susceptible (3, 13, 16, 35), -tolerant (16), -resistant (16), and nutritionally variant (16) viridans streptococcal or staphylococcal (Gavaldà et al., 35th ICAAC) endocarditis models. Based on these results, a successful clinical trial was conducted using the combination of ceftriaxone plus netilmicin, both administered once-a-day during 2 weeks to treat patients with streptococcal endocarditis (14).

The use of combination regimes with aminoglycosides administered once daily for the treatment of enterococcal endocarditis is still controversial (11, 17, 21, 26, 36). Our data suggest that increasing the dosage interval of aminoglycosides to once a day has no influence on the in vivo efficacy of the combination of teicoplanin and gentamicin for the treatment of enterococcal experimental endocarditis, and these data are also consistent with our previous results using a combination of ampicillin and gentamicin once a day for treatment of this disease in rabbits (17). Recently, Schwank and Blaser (36) studied once-daily versus t.i.d. netilmicin combined with amoxicillin, penicillin, or vancomycin against *E. faecalis* in an in vitro model by using H-L. These authors concluded that under their experimental conditions it could not be confirmed that once-daily dosing of aminoglycosides is contraindicated for treating infections caused by *E. faecalis* Moreover, Houlihan et al. (21) compared the pharmacodynamic activities of vancomycin and ampicillin alone or combined with gentamicin given once daily or t.i.d. in an in vitro model of fibrin-platelet clots infected with *E. fecalis*. They found no significant differences in bacterial reductions between the combination regimes of aminoglycosides administered once or thrice daily by using H-L. In con-

TABLE 3. Treatment of experimental endocarditis caused by *E. faecalis* EF91 with a human-like profile with ampicillin or teicoplanin alone or in combination with gentamicin given once or three times a day

Treatment group	No. of animals that died/ total no.	Mean log CFU of vegetation/ g[a] (95% CI)
Control	4/24	9.55 (9.23–9.87)
Ampicillin[b]	3/15	7.25 (6.50–7.99)*
Ampicillin plus gentamicin, tid[b]	2/15	5.23 (4.39–6.07)*†
Teicoplanin[b]	3/18	6.66 (6.15–7.16)*
Teicoplanin plus gentamicin, tid[b]	2/14	4.86 (4.09–5.63)*†‡
Teicoplanin plus gentamicin, once daily[b]	2/14	5.07 (4.22–5.92)*†‡

[a] Results show the mean log CFU of aortic vegetations per gram after 3 days of treatment. The 95% confidence interval (95% CI) is indicated in parentheses. *, $P < 0.001$ versus control; †, $P < 0.01$ versus ampicillin; ‡, $P < 0.05$ versus teicoplanin.

[b] Ampicillin at 2 g/4 h given i.v. (H-L); teicoplanin at 10 mg/kg i.v. (H-L); gentamicin at 1 mg/kg/8 h i.v. (H-L) (gentamicin, t.i.d.); gentamicin at 4.5 mg/kg/24 h i.v. (H-L) (gentamicin, once daily).

trast to these findings and the present and previous (17) results from our group, Fantin and Carbon (11) and Marangos et al. (26) reported that penicillin plus aminoglycoside (netilmicin or gentamicin) given t.i.d. was more effective than penicillin plus the same total daily dose of the aminoglycoside given daily in the treatment of enterococcal experimental endocarditis.

The most probable explanation for the discrepances between our results and those of Fantin and Carbon (11) and Marangos et al. (26) is that the experimental variables in the two studies are distinct, a fact that makes comparison difficult. First, the β-lactams used (pencillin versus ampicillin), although similar, may possess different activities against *E. faecalis* (28). Second, our experimental kinetic model simulated the human kinetics of ampicillin after a 2-g i.v. dose every 4 h A relationship has been shown between the shape of the β-lactam AUCs and their antimicrobial activities (19, 20), this factor may also affect their in vivo efficacy when combined with aminoglycosides. Finally, the doses of aminoglycosides administered t.i.d., in the studies of Fantin and Carbon (11) and Marangos et al. (26) were higher than those used in our studies, which used levels similar to those recommended for the treatment of enterococcal endocarditis in humans (doses that produce peak serum concentrations of 3 to 5 μg/ml) (2). This may explain the slightly increased efficacy (ca. 0.5 $\log_{10}$ CFU/g of vegetation) these authors found with the use of the combinations with aminoglycosides administered t.i.d. If they had used aminoglycoside doses t.i.d., resulting in lower peak concentrations in serum (3 to 5 μg/ml), they might have found an activity similar to the regime of the β-lactam plus the same total dose of aminoglycoside once-a-day. In our opinion, there is no definitive data to date that preclude the use of once-daily administration of aminoglycosides for treating enterococcal infections.

The glycopeptide teicoplanin could be an alternative to a β-lactam for treating enterococcal endocarditis. Teicoplanin has an antibacterial spectrum similar to that of vancomycin and a longer elimination half-life, which allows once-a-day administration by the i.v. or intramuscular route (12). The results of studies with animal models evaluating teicoplanin therapy for enterococcal endocarditis are in accordance with our present study. Sullam et al. (37) reported that teicoplanin was as effective as ampicillin for treating experimental endocardits due to *E. faecalis*. Our results, with use of a human-like kinetic model, are similar to those found in the late 1980s by Sullam et al. In that study teicoplanin alone showed a trend toward higher efficacy compared to ampicillin, though the difference did not reach statistical significance (7.25 versus 6.66 mean log CFU/g of vegetation; $P > to$ 0.05). Likewise, an association with gentamicin given t.i.d. increased the efficacy of teicoplanin alone (6.66 versus 5.07 mean log CFU/g of vegetation; $P < 0.05$). In the study of Chambers and Kennedy (5), the significance of different pharmacokinetic parameters in the efficacy of teicoplanin was examined in experimental endocarditis. The efficacy of telcoplanin was found to be was superior when the drug was administered by the intramuscular rather than the i.v. route, despite the fact that higher peak concentrations in serum were achieved with the i.v. administration. Interestingly, the trough concentration of teicoplanin was higher when it was given intramuscularly (34 ± 10 versus 26 ± 11 μg/ml). These authors concluded that a sustained concentration of teicoplanin in serum several times above the MIC may be important for efficacy in vivo, and they recommended that the trough concentration should be at least 10 times above the MIC of the infecting microorganism. In our study, the administration of the drugs by a computer-controlled pump system provided a teicoplanin trough concentration in serum at 24 h of 21.6 ± 2.9 μg/ml. This concentration exceeds by ca. 20-fold the MBC of teicoplanin for the EF91 strain used in the in vivo experiments (1 μg/ml), and this fact could account for the good outcome of our experiments with the administration of teicoplanin alone or combined with gentamicin.

A small number of patients with *E. faecalis* endocarditis treated with teicoplanin alone have been described in the literature (25, 24, 27, 33, 40). Leport et al. (24) reported one case of enterococcal endocarditis treated successfully with teicoplanin at a dosage of 10.6 mg/kg/day for 48 days. Martino et al. (27) treated four patients; three of them were cured, and one relapsed and was cured with surgery plus treatment with ampicillin and gentamicin. A 4-week course of 10 mg/kg/day has been found to be effective against native streptococcal endocarditis (38). Presterl et al. (33) treated and cured five patients. Lewis et al. (25) reported a retrospective European multicenter study including 115 patients with endocarditis due to gram-positive pathogens, evaluating the outcome: efficacy, and safety of using teicoplanin alone or in combination with other drugs. There were 22 cases of enterococcal endocarditis: 10 treated with monotherapy and 12 treated in combination with aminoglycosides. All of the patients treated with the combination were cured, whereas treatment was not successful in 2 of the 10 patients treated with monotherapy. Despite these promising finding, it seems reasonable to combine treatment with aminoglycosides if teicoplanin is used for the treatment of enterococcal endocarditis.

Home treatment would potentially be of benefit in endocarditis because therapy is prolonged, and the patients are frequently capable of self-care after the first 2 weeks of treatment. One important area of research interest is single daily-dose regimens that could allow home therapy for selected cases of endocarditis, with attendant cost savings. In studies of experimental streptococcal endocarditis, a single-daily dose of an aminoglycoside in combination with ceftriaxone was as effective as the elective treatment with penicillin plus aminoglycosides in divided doses (3, 13; Dorscher et al., 30th ICAAC). Teicoplanin plus gentamicin, both administered once a day, could be an alternative therapeutic approach for selected cases of enterococcal endocarditis on an outpatient basis, probably after 2 weeks of treatment in the hospital environment. Our experimental results showed that teicoplanin plus gentamicin, both administered once a day by means of a controlled infusion pump system to mimic human kinetics, was as effective as the elective treatment with ampicillin at 2 g/4 h plus gentamicin at 1 mg/kg/8 h. The results from a recent clinical study by Venditti et al. (38) provide evidence that a single daily dose of teicoplanin plus gentamicin could be a good therapeutic option for home treatment of selected cases of enterococcal endocarditis. Six patients with enterococcal endocarditis were cured when treated with teicoplanin in combination with gentamicin or netilmicin administered once a day.

In conclusion, the efficacy shown in our experiments with the combination of teicoplanin and gentamicin, both administered once a day, indicates that this combination could be valuable in

the treatment of enterococcal endocarditis and may be useful as home therapy in cases of uncomplicated enterococcal endocarditis, reducing the time and cost of hospital care. Further studies are needed to establish the real efficacy of this therapeutica approach in humans with enterococcal endocarditis.

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